

Title: RENILLA REINFORMIS FLUORESCENT PROTEINS,
NUCLEIC ACIDS ENCODING THE FLUORESCENT
PROTEINS AND THE USE THEREOF IN
DIAGNOSTICS, HIGH THROUGHPUT SCREENING
AND NOVELTY ITEMS.

Applicant: Bryan et al. Our Docket No.: 24729-0128
Serial No. 09/808,898 Filed: March 15, 2001

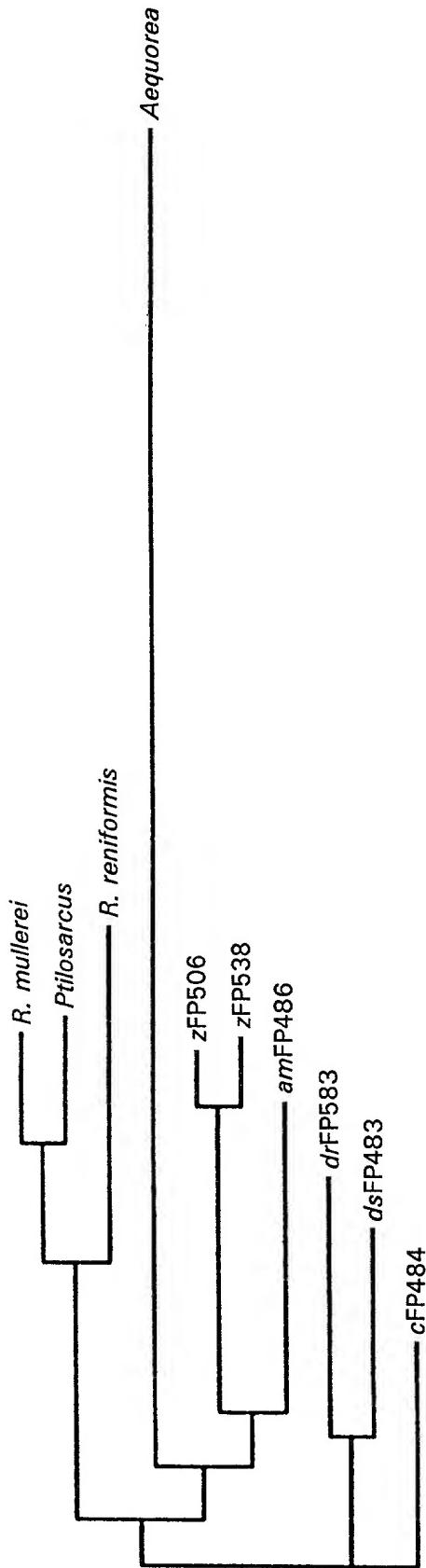


FIG. 1

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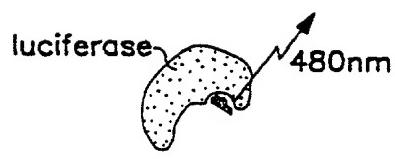


FIG. 2A

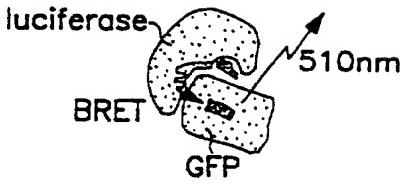


FIG. 2C

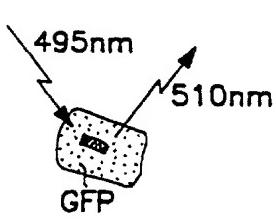


FIG. 2B

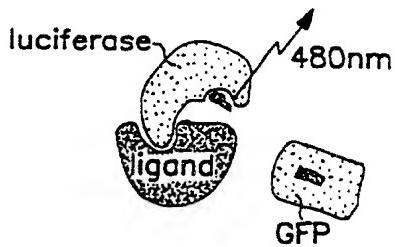


FIG. 2D

BRET Sensor Architectures

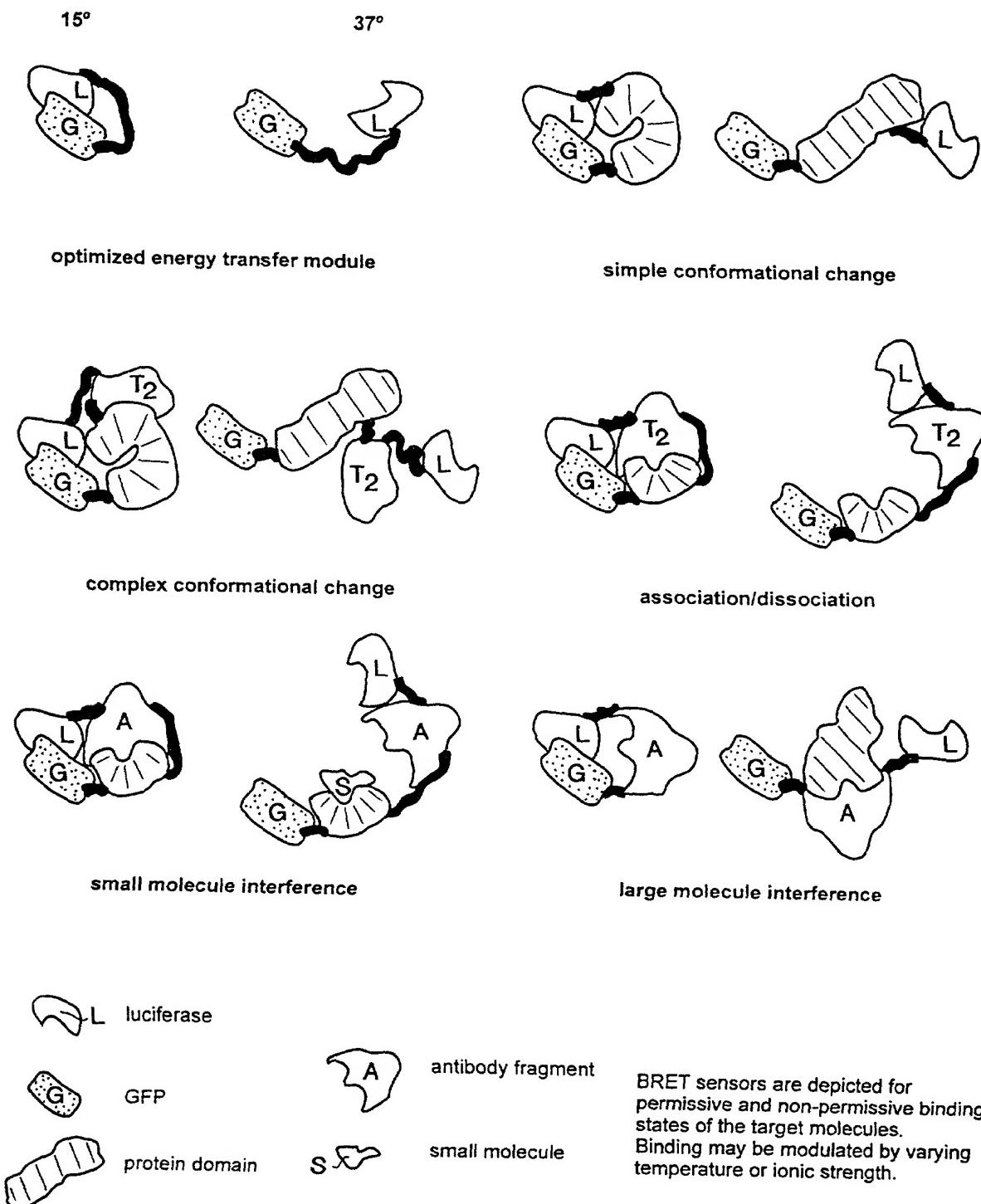


FIG. 3

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Utilization of advantageous GFP surfaces with
 substituted fluorophores

	60	*	80	
RM-GFP	:	GAPLPFAFDIVSPAFQYGNRTFTKYPNDIS--		: 83
Pt-GFP	:	GGPLPFAFDIVSIAFQYGNRTFTKYPDDIA--		: 83
RR-GFP	:	GAPLPFAFDIVSVAFSYGNRAYTGYPEEIS--		: 80
cFP484	:	GAPLPFSYDILSNAFQYGNRALTKYPDDIA--		: 83
drFP583	:	GGPLPFAWDILSPQFQYGSKVKHPADIP--		: 80
asFP595	:	GGPLPFAFHILSTSCMYGSKTFIKYVSGIP--		: 77
dsFP483	:	GGPLPFGWHILCPQFQYGNKAFVHHPDNIH--		: 80
amFP486	:	GGPLAFSFIDILSTVFKYGNRCFTAYPTSMP--		: 82
zFP506	:	GGPLPFAEDILSAAFNYGNRVTTEYPQDIV--		: 80
zFP538	:	GGPLPFSEDIILSAGFKYGDRIFFTEYPQDIV--		: 80
=====				

FIG. 4

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R_reniform	---	MDLA KGLKEV MPTKINLEG VCGD HAFSME GVGE GNILEGT QEVVKIS VTKGAPL FADFIVSV	*	*	60
R_mullerei	:	M SKOJ.I.KNTC.Q. SY.V. I.NN.V. I.NN.V. I.NN.V. I.NG.E.	*	*	20
Ptilosarcu	:	M NRNV.KNT. SA.ASV. I.RF.VRM. I.FI.E.	*	*	40
drFP583	:	---	*	*	60
	X	X	X	X	X
R_reniform	:	A FSYGNRAYTGY PEETSDY FFLQS SFPEEGFTY ERNRIRYQ DGGTA IVKS DISIEDGKF FLVN VDFKA KDL	*	*	80
R_mullerei	:	Q TF.K. ND I.D. TF.K. SKV.VKH.	*	*	100
Ptilosarcu	:	Q TF.K. D.A. V. A. KHL	*	*	100
drFP583	:	Q Q. AD.P. X	*	*	120
	X	X	X	X	X
R_reniform	:	RRMGPVMQ QDIVGMQPS YE SMY TNTVS VIGEC IAFKI QTGKHT YHMRTV YKSR KPVET MPL YHF	*	*	140
R_mullerei	:	PDD KT.L.IE F.A.M. NGVLV V.LVY NS YYSC K.LM GV KEF S..	*	*	160
Ptilosarcu	:	PSN KA.L. E. V.V.M. SGVLV VDLVY ES NYYC K.F.R. GG KEF E..	*	*	180
drFP583	:	PSD KKTM. WEA. T.RL. PRDGVLK IHK.L.	*	*	200
	X	X	X	X	X
R_reniform	:	IQRHIVKT VNDTAS GYVQ HETATIAAH STIKR IEGSTIP ---	*	*	00
R_mullerei	:	...E.Y. EDGGF -E. ...H..E..Y. EEG.F.-E. VDSK.DI.	*	*	220
Ptilosarcu	:	...OMTS.G. PL...HEWV QLT..G.PL...HEWV SHNEDYTI.E.Y. RRIEGR.HLFL	*	*	233
drFP583	:	---	*	*	238
	0	0	0	0	0

5
FIG.

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Aequorea	* 20	* 40	* 60	* 80	
R_mullerei	--MSKGEELFTGIVPILVLLDGVINGHKFSSVSGEGEQDAITYGKTLIKEFCTT--	--GKLFVVPWPTLVITTSYGVQCFSRYPDDMK	--		79
Ptiosarci	--MSKQIQLNTCLQEVMSYKVNLGIVNNHFTMEEGCCGNILFGNQLVQIRVTK--	--GAPLFAFDIVSPAFQYGNRFTKYPNDI-	--		82
R_reniform	--MNRNVNLKNTGLKEIAMSAKASVIEGIVNNHFTSMEGFKGNVILFGNQLMQIRVTK--	--GGPLFAFDIVSVAFAQYGNRFTKYPDDI-	--		82
drFP583	--MDLAKLGLKEVMPPTKINLEGLVGDHA	--GAPLFAFDIVSVAFAQYGNRATGYYPEEI-	--		79
drFP593	--MRS5KVNKEFMRFKVMEFFIEGEGRPEYEGHCSVKLMVTK--	--GGPLFAFDILSPOFQGSKVKYPADI-	--		79
dsFP483	--MSCSKVNIKEFMRFKVMEFFIEGEGRPEYEGHCSVKLMVTK--	--GGPLFAFDILSPOFQGSKVKYPADI-	--		79
cFP484	--KALTIGVIKPKDMKIKLKME	--GGPLFAFDILSPOFQGSKVKYPDDI-	--		82
asFP595	--MASFLKKTMPFKTTLIEGIVNGHAFVIEGECKPKYDGTHTNLLEVMAEGAPLPFSYDILSNAFOQNRAUTKYPDDI-	--GGPLFAFDILSPOFQGSKVKYPGGI-	--		76
amFP486	--MALSNKFIDDDMKMTYHMDGCIVNGHYFTVKGEENGKPYEGTQQTSTFKVTMANGPLAFAESFDILSTVFKYGNRCFTAFTPSM-	--GGPLFAFDILSAGFKYGDRLIFTEYQDIDI-	--		81
zFP538	--MAHSKHGLKEEMTMKYHMEGCIVNGHKFVITGEIGYGPFKGKQ--	--TINLCVIEGGPLPFSEDLILSAGFKYGDRLIFTEYQDIDI-	--		79
zFP506	--MAQSKHGLTKEMTMKYRMEGCIVDGHKFKVITGEIGYGPFKGKQ--	--AINLCVVEGGPLPAEDILSAAFNIGNRVRFTFEYQDIDI-	--		79
* 100 * 120 * 140 * 160 *					
Aequorea	RHDFFKSAMPEGYQVQERTIFFEDGNYKTRAEVKFEG--	DTLVNRTELKGTDKEDGNILGHKLEYNNNSHNVYTMADKQKNGJK	--		162
R_mullerei	-SDYFIQSFPAGFMERTLRYEDGGLVETRSDINLIE--	-DKFVYVTRVETKGSNFPPDGPMQKTTI-LGIEPSFTEAMYM--	-NNGVIV	--	161
Ptiosarci	-ADYFVQSFPAFFFYERNLRBEDGAIATYDIIERSDISLED--	-DKEHYVCKVEMRGNFFPSNGPVMQKAI-LGMEPSFEEVYMM--	-NSCVIV	--	161
R_reniform	-SDYFLQSFPPEGFTYERNLRYQDGGIAIVKSDISLED--	-GKPFIVNWDFAKDLRRMGPVMQQDI-VGMQPSTSIESMT--	-NVTSVI	--	158
drFP583	-PDYKKLSFPEGFKWERVINPFDGGVWMTQDSSLQD--	-GCFIYKVKREFIGVNFPSDGPVMQKKT-MGWESTERLYP--	-RDGVWK	--	158
drFP593	-PDYKKLSFPEGFKWERVMNFDGGUNIVSQDSSLKD--	-GCFIYEMKFLIGVNFPSDGPVMQRRT-RGWEASSERLYP--	-RDGVWK	--	158
dsFP483	-HDYKLKLSFPEGTYWERSMHRBEDGGLCCITNDISLTG--	-NCFYYDTIRETGYLNFPTINGPVPVQKKT-TGTEPSTERLYP--	-RDGVWI	--	158
cFP484	-ADYFQKSFPPEGYSWERTMTBEDGKLYVKVSKDISMSEE--	-DSFITYEIRFDGMNPFPNGPVMQKKT-LKWEPSTEIMYV--	-RDGVLY	--	161
asFP595	-PDYFKQSFPPEGFTWERTITYEDGGFLTAHQDTSDLG--	-DCLVYKVKILLGNNFPADGPVMQNKAA-GREWPAEIVYE--	-VDGVIR	--	155
amFP486	-PDYFKQAFFDGMYSYERTFTYEDGGVATASWEISLKG--	-NCFEHKSTIFHGVMNPADGPVMKKT-TGWDPSFEKTMV--	-CDGILK	--	160
zFP538	-VDYFKNSCPAGYTGRSFLIEDGAVCINCVIDTVSKYKSHIFNGMNPADGPVMKKT-TNWEASCEKIMPVPKQGILK--	--			162
zFP506	-VDYFKNSCPAGYTGRSFLIEDGAVCINCADITVSVEENCMYHESKYFVYMFADGPVMKKT-DNWEPSCKEILPVPKQGILK--	--			162
* 200 * 220 * 240 *					
Aequorea	VNFKTRHNIEDGSSVQFADHYQONTPIG-DGPVLLPDNTHYLSTQALSKDPNKRDHMVLLEFVTAAGITHGMDELYK---	--			238
R_mullerei	GEVILMVKLNSGKNTYISCHMKTIMSKG--	--VVKEFPSYHFIQHRLKETYVEDGCF--	-VEQHETAIACMITSIGKPLGSLHEWV	--	238
Ptiosarci	GEVDLVYKLESGNYSCHMKTIVRSKG--	--GVKEFPEYHFIHRLKETYVEGSF--	-VEQHETAIACLTIGKPLGSLHEWV	--	238
R_reniform	GECITIAFKLQLTGKYYTYHMRTVYKTSKK--	--PVETMPLHYFIQHRLVYKTNVDTA5G-YVVQHETATAAHSTTRKIEGSLP--	--		233
drFP583	GEIIEKALKLKDGGHMLVEFKSISIYMARK--	--APVQLPGCYYYVDSKLDLJISHNEDYT-IVEQYERTEGRHIELI--	--		226
drFP593	GDIEHMLRLEGGGHMLVEFKSISIYMKK--	--PSVQLPGCYYYVDSKLDLJISHNEDYT-VVEQYKETQGRHFPIKPLQ--	--		230
dsFP483	CDIEHHLALTVEGGHHAACDIKTVTRAKK--	--AALKMPGHYIYDITKLVWNNDKEFM-KVEEHEDIAVARHPPTEPKKDK--	--		232
cFP484	CDIISHESSLLEGGGHHRCDFSIYKAK--	--KVKVLDPDYHFDHRIEILLNHDKDN-KVTLVNEAVARYSILPSQA--	--		231
asFP595	CQSLIMALKCPGGRHILTCHLHTTYRISKPKASA--	--LCKMPGFHEDHRIEIMEYEKGK-CYKQYEAAVGRYCDAAAPSKLGHN--	--		232
amFP486	GDVTAFLMLQGGGNYRCQFHTSYKTK--	--KPVTMPPNHWVEHRTARTDLDKGGN-SVQLTEHAVAHTITSWTF--	--		229
zFP538	GDVSMVLLLDGGRYRCQFDTWYKAK--	--SVPSKMPPEMFIQHKLJREDRSDAQNQKWLTEHATAFPSALA-----	--		231
zFP506	GDVSMVLLLDGGRYRCQFDTWYKAK--	--SVPRKMPDWFHQHKLJREDRSDAQNQKWLTEHATAFSGALP-----	--		231

D,E,H,K,R	N,Q,S,T	L,I,V,M,F,Y,W	A,G	C,P
polar charged	polar uncharged	non-polar hydrophobic	small	not grouped

dimerization	<input type="checkbox"/>	hydrophilic
surfaces	<input type="checkbox"/>	hydrophobic

FIG. 6